

Nutrient-water fluxes and uptake along the midgut of *Zonocerus variegatus* (L.)(Orthoptera : Pyrgomorphidae)

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Abstract : Circulation of water and nutrients in the midgut of *Zonocerus variegatus* was studied to determine the flux pattern and its possible implication in the adaptation of the insect to hydrogen cyanide. Histological sections of midgut cells were observed. Gradients of K^+ , Na^+ and protein along midgut were determined. The permeability of midgut segments to K^+ , Na^+ , Ca^{2+} , Mg^{2+} and methyl blue was observed. The midgut(ventriculus and gastric caeca) was histologically similar , and made of columnar cells with striated borders. The nutrients varied in concentration in the different midgut segments. The substances tested for permeability passed through the midgut segments. It was concluded that the entire midgut was involved in food and water absorption. There was no counter flow of fluids from the posterior midgut as in most orthopterans. One-way movement of the gut contents prevents accumulation of toxic substances to injurious levels , enabling the insect to favourably tolerate hydrogen cyanide in cassava leaves.

Key words : *Zonocerus variegatus* ; midgut ; nutrient ; water ; permeability ; fluxes ; uptake

1 INTRODUCTION

The circulation and uptake of water and nutrients along insect midgut has been severally studied. Treherne (1958a , 1958b , 1958c) using *Schistocerca gregaria* and *Locusta migratoria* reported that it is the anterior gastric caeca which is the main site for food uptake. Berridge (1970) confirmed this assertion and added that absorption by the caeca is aided by a flow of fluid from the posterior midgut which washes the nutrients upstream into the anterior caeca. Dow (1981b) suggested that this fluid was Malpighian tubule discharge which some part flows anteriorly. Ferreira *et al.* (1981) further confirmed this counter-current idea by identifying the posterior ventriculus of *Rhynchoscaria americana* as being histologically differentiated for fluid secretion. Reports so far have classified insects into three putative ancestral groups (Panorpoid , Neoptera and Holometabola ancestors)(Terra , 1990). Other specialized insects are believed to have evolved from these. Available reports of the circulation of materials in orthopterous insects ' guts concern *S. gregaria* (Treherne , 1958a , 1958b , 1958c ; Dow , 1981a) and *L. migratoria* (Baines , 1979 ; Dow , 1981b). This then gives the impetus to find out how materials circulate along other orthopterous guts , e.g . *Zonocerus variegatus* . This insect is a polyphagous pest feeding mostly on cassava leaves and there has been no satisfactory explanation of its adaptation to hydrogen cyanide (HCN) , apart from the reports of Bellotti *et*

al. (1999). It is therefore expected that this study will help to explain further the adaptation of *Z. variegatus* to HCN. The study is therefore designed to include histological studies of the midgut cells , nutrient gradients along the midgut and permeability of the midgut epithelium to stains and cations .

2 MATERIALS AND METHODS

2.1 Histology

Fifty adult male and female *Z. variegatus* were dissected and guts were removed and fixed immediately in Bouin fluid (Pantin , 1964) for 24 h. The midgut was processed for microscopic examination according to standard histological procedures. All sections were observed under light microscope at 10 × and 40 × magnifications .

2.2 Material gradient

Nutrients and solute gradient studies in K^+ , Na^+ , and protein (nitrogen) in crop , ventriculus , gastric caeca and hindgut were carried out. Dissected out (not excised) guts were ligatured into separate midgut regions and gut fluids extracted from each region with microsyringe. Extracts from each region were separately analysed photometrically using Flame Photometer. Protein concentration was determined using micro Kjeldahl method (AOAC , 1996).

2.3 Midgut permeability to methyl blue stain

Dissected out (not excised) gut of *Z. variegatus* adults were ligatured at separate points into distinct

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regions (anterior , and posterior midgut , anterior and posterior gastric caeca). They were immersed into a known concentration of methyl blue stain for 30 min . At the end , the insects were removed and properly rinsed and dried with filter paper to remove excess methyl blue from the surface. Lumen content of each section was drawn with microsyringe. A known volume of each extract was diluted with equal volume of distilled water. Concentration of methyl blue in the extract was determined photometrically.

2.4 Midgut permeability to K^+ , Na^+ , Mg^{2+} and Ca^{2+}

Guts of fed insects were dissected out and each region was separately ligatured and placed in a small bottle (100 cc) containing 3 mL of deionized water and allowed to remain for 1.5 h before the gut region was removed. Care was taken such that no part of the gut was cut or punctured. The deionized water specimens were photometrically analysed at IITA in Ibadan using Atomic Absorption Spectrophotometer , Unicam 919 , for each of the ions.

Crop segment of the gut was used as control for the permeability studies.

3 RESULTS

3.1 Micromorphology

The epithelium of the midgut showed a single layer of columnar cells over a muscle layer. The cells had striated luminal surfaces. Their nuclei varied in shapes sizes , position and staining. Based on these variations , the epithelial cells were categorised as regenerative , young , resting , extruding and erupting (Akpan and Okorie , 2005). A section of the midgut too showed the peritrophic membrane(Plate I :1) extending from the cardia through the ventriculus into the hindgut.

3.2 Material gradient

Potassium and sodium concentrations varied inversely along the insect gut. Potassium increased in concentration from the posterior gastric caeca and was greater in the posterior midgut ; the least concentration was observed in the anterior caeca(Fig. 1). Sodium content was greatest in the posterior caeca and least in the hindgu(Fig. 1). Protein was present in all regions of the insect gut. The highest quantity was found in the anterior midgut and the least quantity in the crop. Its concentration in other gut regions varied between 1.3 and 1.8 $\mu g/L$ (Fig. 2).

3.3 Permeability of midgut to methylene blue

Methylene blue was absorbed by the midgut epithelium from the medium into the gut lumen (Table 1). Posterior midgut , anterior caeca and posterior caeca absorbed far more than the anterior midgut 30 min after specimens were immersed in the methylene blue.

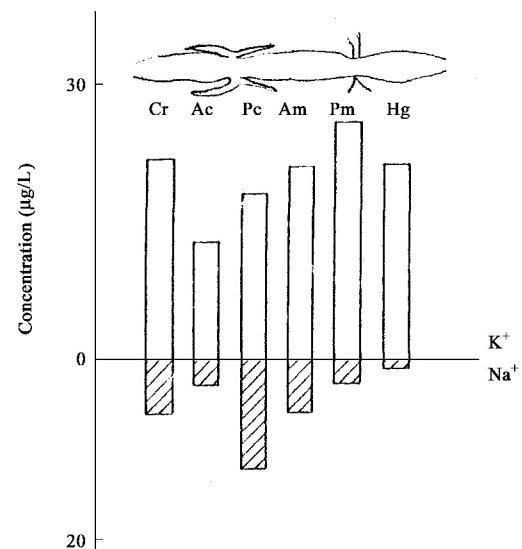


Fig. 1 The distribution of K^+ and Na^+ in *Z. variegatus* gut with gut regions where samples were taken superimposed
Cr : Crop ; Ac : Anterior caeca ; Pc : Posterior caeca ; Am : Anterior midgut ; Pm : Posterior midgut ; Hg : Hindgut. The same below.

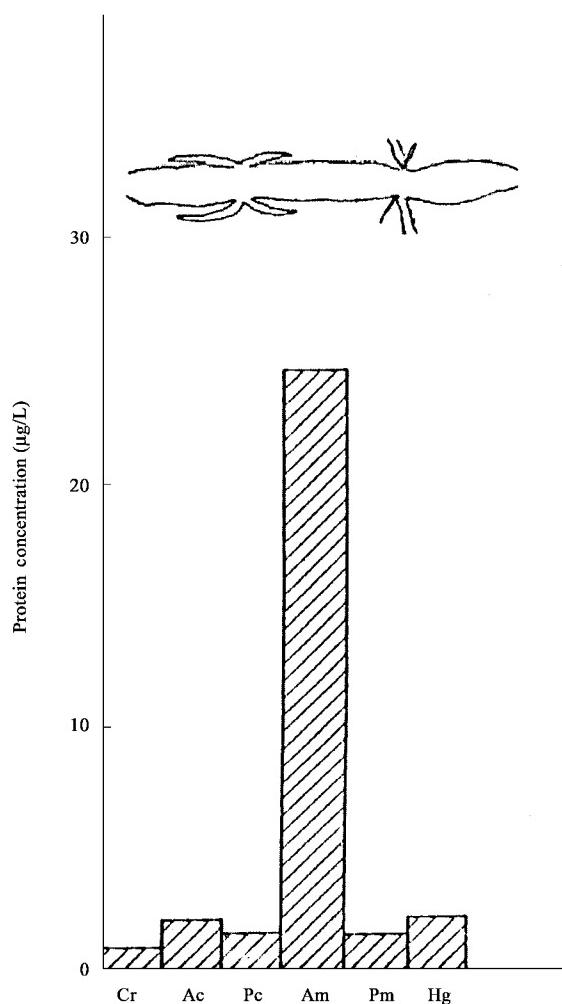


Fig. 2 The distribution of protein molecules along *Z. variegatus* gut with gut regions where samples were taken superimposed

Table 1 Absorption of methylene blue by the midgut

Time exposed (min)	Amount of methylene blue absorbed ($\mu\text{g/L}$)			
	Anterior midgut	Posterior midgut	Anterior caeca	Posterior caeca
30	10.00 \pm 7.07	250.00 \pm 30.33	321.00 \pm 9.18	175.00 \pm 17.21
60	415.00 \pm 41.23	532.00 \pm 32.23	472.00 \pm 37.23	219.00 \pm 17.42

3.4 Permeability of midgut to cations

The four cations tested permeated the various segments of the midgut into the deionised water (Table

2). Anterior midgut and anterior gastric caeca were more permeable than other midgut segments.

Table 2 Permeability of midgut to cations

Gut region	Cations and the amount (ppm) passed into deionised water			
	K ⁺	Na ⁺	Mg ²⁺	Ca ²⁺
Anterior midgut	84.05 \pm 5.98	6.68 \pm 0.98	101.43 \pm 1.64	49.99 \pm 4.26
Posterior midgut	65.41 \pm 3.54	6.38 \pm 0.69	12.96 \pm 1.08	10.42 \pm 1.54
Anterior caeca	104.82 \pm 9.66	14.27 \pm 1.30	61.12 \pm 21.29	38.22 \pm 4.68
Posterior caeca	37.04 \pm 5.45	8.89 \pm 0.74	17.77 \pm 1.38	10.52 \pm 1.44

4 DISCUSSION

The midgut (ventriculus and gastric caeca) of *Z. variegatus* has a uniform epithelium with a layer of columnar cells with striated border, on a basement over a muscle layer, as in most insects (Khan, 1964; Gouranton, 1968; Smith *et al.*, 1969). The epithelium of the anterior caeca has folds with crypts between the folds. The posterior caeca has numerous unlocalised crypts (Akpan and Okorie, 2005). The folds and crypts are suggested to be devices for increasing the surface area of the caeca for their functions. The presence of brush border has been identified as a common characteristic of digestive and absorptive cells. This is because most digestion and subsequent absorption take place at the brush border of the cells (Smith *et al.*, 1969). Since there were no distinct morphological differences among the midgut cells (apart from the differences relating to their ages), the cells were concluded to be involved in digestion and absorption of food. Similar observations in *Locusta* were made by Baines (1979) who concluded that there were no macroscopic differences observed between absorbing and non absorbing midgut cells. Insects with functional differentiation have cells that differ morphologically to suit their function (Gillot, 1980; Ferreira *et al.*, 1981; Volkmann and Peters, 1989).

The peritrophic membrane (PM) as shown on Plate I (1) is common in *Z. variegatus* as it is among other solid feeders. It extends from the oesophageal invagination across the caecal opening into the ventriculus without entering the caeca. Functions of this membrane had been discussed by Wigglesworth (1967), Terra *et al.* (1979), Bernays and Chamberlain (1980), Terra (1990), Barbehenn and Martin (1992), Felton and Summers (1995) and Lehane (1997). Its function relevant to this study is its

serving as a visceral filter, filtering food monomers and dimers into the ectoperitrophic space, and enhancing uptake of the digested particles by the epithelial cells into the haemolymph. The role of the tubule fluid in filtering the food monomers upstream for absorption becomes less significant. The presence of the PM provides a steady medium into which digested food diffuse and are brought closer to the cell surfaces. The movement of materials from the endospace into the ectospace of the membrane is supposed to be assisted by the unstirred layer around the membrane (Lehane, 1976) which allows movement of materials mostly in one direction.

Although the functions of this membrane had been discussed, it could be useful in faeces formation by arranging the undigested mass into a cylindrical shape that can be separated at the ventricular hindgut junction. In some orthopterans, e.g. *Schistocerca* (Goodhue, 1963), faeces formation is done by the S-shaped colon, which is absent in *Z. variegatus*.

The accumulation of materials along the insect midgut is shown to be contradictory to other ideas considered common to orthopterous insects. It shows that there is no counter-current in *Z. variegatus* midgut. High concentrations of potassium (the prime water mover) at posterior midgut than anterior midgut can not draw water into anterior midgut and caeca to cause counter flow. For counter-current to occur, the system should produce a standing gradient of solutes preferably at a higher concentration in the anterior midgut than the posterior midgut (Dow, 1981b).

The permeability studies of the ventricular and caecal epithelia show that materials such as water, ions and food monomers can cross the epithelia into the haemolymph and vice versa (Dow, 1986). It is considered that food and water are absorbed by the cells into the haemolymph and water is withdrawn continuously again from the haemolymph into the gut

lumen for food digestion and uptake. Hence water pathway is formed transversely between the gut and haemolymph through the epithelium. The food absorbed is converted immediately according to Terrunno (1984) into trehalose and stored in fat bodies thus creating a gradient for continuous food uptake. Counter-current system cannot be confidently inferred in this study for *Z. variegatus*. Results of permeability studies in this case have vividly shown the pattern that food, water and other materials circulate between the lumen and haemolymph across the epithelium.

In *R. americana*, the counter flow idea is clearly explained by Ferreira et al. (1981). Here there is a clear-cut histological and physiological distinction of the midgut into compartments. But such organization is not observed in *Z. variegatus* midgut. The homogeneity of

the midgut microstructure shows that the same cells are involved in the secretion of digestive enzymes and absorption of food (Akpan and Okorie , 2003 , 2005).

The pattern of circulation of materials (material pathway) in *Z. variegatus* midgut differs remarkably from the three fundamental ancestral patterns (Terra , 1990). In these three groups , the direction of circulation is presumed to be anterior-posterior. In *Z. variegatus* , circulation of water and nutrients is proposed to be trans-membrane trans-epithelia(crossing the peritrophic membrane and epithelium between the lumen and haemolymph(Fig. 3). This trans-membrane trans-epithelia pathway could be referred to as the Zonoceroid model. This presumably has some advantages over the putative ancestral patterns discussed.

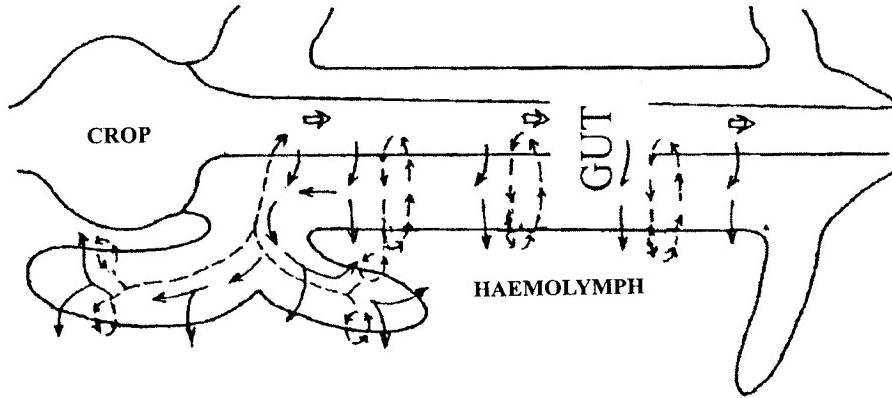


Fig. 3 Diagrammatic representation of water(dotted arrows) and nutrients(solid arrows) circulation in *Z. variegatus* midgut. The open arrows indicate direction of food movement.

First , this model prevents silting of the anterior regions and accumulation of plant toxins. Secondly , it reduces interference with the downstream movement of the gut content which should retard digestion and feeding whereas the insect is known to feed voraciously (Peacock , 1913). According to Fried et al. (1986) counter-current flow prolongs digestive periods in insects which present a great disadvantage to this insect. Thirdly , the suggested pattern pushes accumulated materials on the cell border into the lumen as the cells secrete the fluid(Plate I : 2). There is a lifting of a layer of materials from the epithelial surface into the lumen. This could be what Dow (1981b) referred to as the " garbage disposal " , and caecal emptying serves as means of unsilting the blind end sacs (caeca). Clogging of the brush border can interfere with the surface activities of the cells . Finally , cycling of water and nutrients flow between the gut lumen and haemolymph would follow a straight route across the entire midgut epithelium. This makes digestion and absorption proceed quickly , hence high consumption .

The most important advantage of this flux model is that it offers the insect an opportunity for polyphagous life. Polyphagy exposes the insect to different

allelochemicals which vary in number according to the number of plant species the insect feeds on. Some of these substances have immediate poisoning effects , e.g. hydrogen cyanide (HCN). Generalist feeders such as *Z. variegatus* have preference for low HCN (Bellotti et al. , 1999), which means the lower the concentration , the less potential its harm to the insect. It could then be presumed that as the food (gut content) is passing downstream , without counter-current , there will be no upstream wash. As such , there will be no accumulation of the poisoning substances anteriorly to concentrations that can become injurious to the insect. Therefore these substances pass through the gut in small titres that could be tolerated by the insect. It therefore becomes an adaptation to the insect enabling it to feed on cassava leaves. Cassava plants with low cyanide contents are more vulnerable to the insect attacks than the varieties with high cyanide content. Logically cassava plants with high cyanide contents will not be preferable to the insect because it lacks a detoxifying mechanism.

Studies have been carried out by Modder (unpublished) , Oladapo (1979) and others to determine the mechanism the insect uses to overcome

these toxic substances. Chapman *et al.* (1986) reported that the adaptation of this insect to HCN is uncertain. It is generally known that the insect tolerates low titres of HCN and prefers cassava leaves, especially senescent leaves, of low HCN (Chapman *et al.* , 1986 ; Bellotti *et al.* , 1999). But how the HCN concentration is reduced to the insect 's tolerance has never been explained. In application, the production of high HCN cassava hybrids which will become unpalatable may conveniently reduce the feeding intensity and subsequent pest impact reducing the need for pest control.

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臭腹腺蝗(直翅目：锥头蝗科)中肠内营养物-水分的流动和吸收

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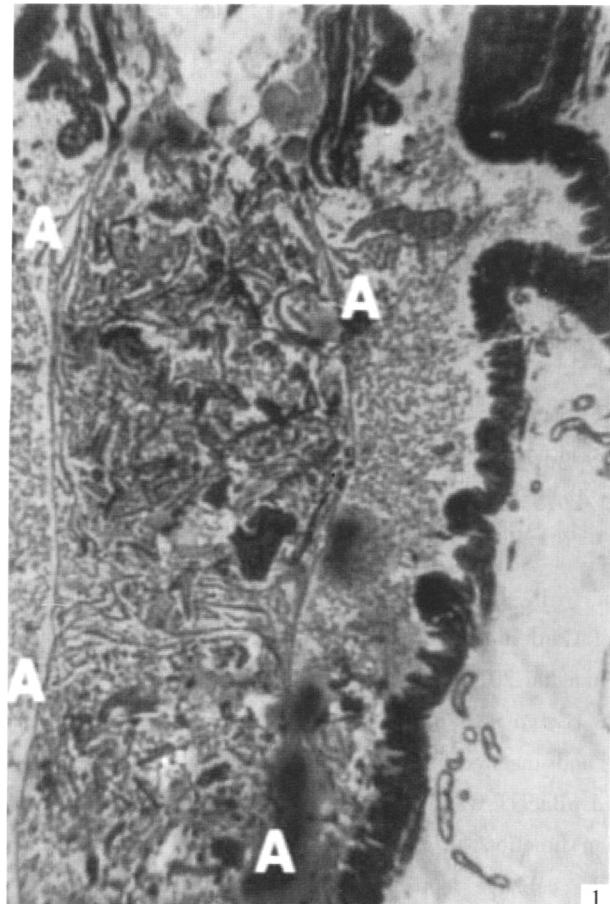
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摘要：对臭腹腺蝗 *Zonocerus variegatus* 中肠内水分和营养物的流动和吸收进行了研究,以期确定其流动模式及在该虫对氰化氢适应方面的意义。对中肠细胞进行了组织切片观察,测定了K⁺ ,Na⁺ ,Ca²⁺ ,Mg²⁺ 和甲基蓝的通透性。结果表明,中肠(胃和胃盲囊)组织上结构相似,均由具有纹状边缘的柱状细胞构成。营养物质在中肠不同部分的浓度不同。测试物在整个中肠中流动,中肠各段均参与了食物和水分的吸收。臭腹腺蝗肠内没有像大多数直翅目昆虫那样自后肠的液流逆向流动。肠溶物的单向流动可以防止有毒物质的积累,使臭腹腺蝗成功耐受木薯叶中的氰化氢。

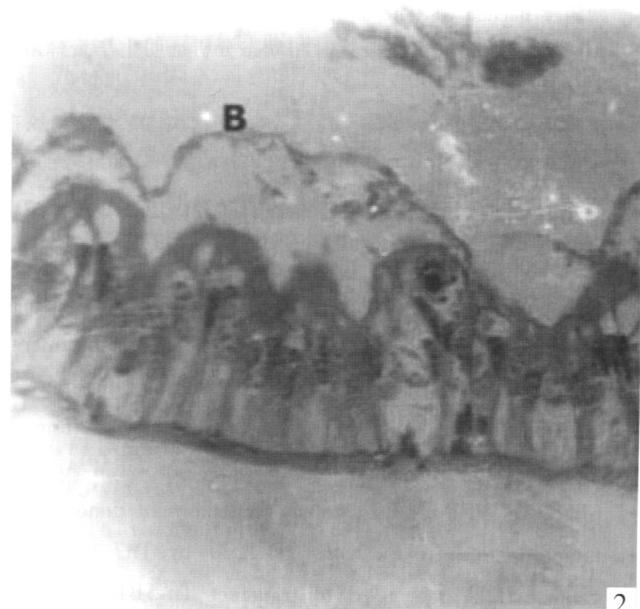
关键词：臭腹腺蝗 ; 中肠 ; 营养 ; 水分 ; 通透性 ; 流动 ; 吸收

中图分类号: Q965 文献标识码: A 文章编号: 0454-6296(2006)01-0010-06

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2

Microstructure of the midgut of *Zonocerus variegatus*

1. Longitudinal section of the ventriculus showing the peritrophic membrane (letter A);
2. Longitudinal section of the posterior gastric caeca showing the peritrophic membrane (letter B) lifted from the surface of the epithelium.